Remarks

Reconsideration of this Application is respectfully requested. Upon entry of the foregoing amendment, claims 1 and 67-83 remain pending in the application, with claims 1 and 78-83 being the independent claims. The specification has been amended herein to include an abstract on a separate page. The specification has also been amended to direct the entry of a sequence listing at the end of the captioned application and to provide the SEQ ID NO's next to the specific sequence. In accordance with 37 C.F.R. § 1.821(g), this submission includes no new matter. In accordance with 37 C.F.R. § 1.821(f), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith in the above application are the same. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicant respectfully requests that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Objection to the Specification

The abstract was objected to because "it is merely a copy of the first page of the corresponding PCT. A clean abstract on a separate sheet of paper is required." Office Action, page 2, lines 8-10. Filed concurrently herewith is an abstract on a separate sheet, and an amendment directing its entry. Thus, this objection has been accommodated and it is respectfully requested that it be withdrawn.

Double Patenting Rejection

The Examiner has made a provisional double patenting rejection under 35 U.S.C. § 101. Office Action, page 2, line 12, through page 3, line 4. In particular, claims 1 and 67-83 are rejected as being in conflict with claims 1-13, 27, 53, 55, 57 and 60 of co-pending Application No. 10/391,216 ("the '216 application"). Applicant respectfully traverses the rejection.

Applicant submits that the rejection is in error because claims 1-13, 27, 55, 57 and 60 are not pending in the '216 application. Applicant filed a preliminary amendment in the '216 application on March 19, 2003, canceling claims 1-52 and 54-66. A subsequent preliminary amendment filed on December 19, 2003 shows that only independent claim 53 and its dependent claims 67-86 are pending in the '216 application. Enclosed are copies of these preliminary amendments and their respective postcards, date-stamped by the U.S. Patent and Trademark Office as having received on March 19, 2003 or December 19, 2003 the documents listed thereon.

The Examiner's rejection of the pending claims of the captioned application in light of claim 53 of the '216 application is also improper. Claim 53 of the '216 application is directed to a method for providing a mammal a prophylactic or therapeutic treatment associated with a bacterial infection comprising administering to the mammal an immunogenic composition comprising one or more immunogen-encoding polynucleotides associated with the bacterial infection and an adjuvant composition comprising GAP-DMORIE and a co-lipid, wherein an immunogen is expressed in the mammal in an amount sufficient to generate an immune response to the immunogen. Hence, claim 53 and its dependent claims are directed to a *method* comprising

administering an immunogenic composition comprising *one or more immunogen-encoding polynucleotides*.

In contrast, none of the claims of the captioned application are directed to such a method. Pending claim 1 and its dependent claims 67-77 are directed to an adjuvant composition; claim 77 is directed to an immunogenic composition; and claim 83 is directed to a pharmaceutical kit. Independent method claims 79-82 are each directed to a method comprising administering an immunogenic composition comprising, *inter alia*, one or more antigenic *polypeptides*, immunogenic *polypeptides* or *polysaccharides*. Hence, none of the pending claims of the captioned application are directed to a method comprising administering an immunogenic composition comprising *one or more immunogen-encoding polynucleotides*.

As the claims of co-pending Application No. 10/391,216 and the claims of the present application are not directed to identical subject matter, the provisional rejection under 35 U.S.C. § 101 is improper. Accordingly, Applicant respectfully requests that the Examiner reconsider and withdraw the rejection, and place the claims in condition for allowance.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Aaron L. Schwartz
Agent for Applicant
Registration No. 48,181

Date: May 14, 2004

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

240275_1.DOC

OIPE JC177
MAY 14 2004
PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
Carl J. Wheeler
Appl. No. (To be assigned)
Filed: (Herewith)
For: **Adjuvant Compositions and Methods for Enhancing Immune Responses to Polynucleotide-Based Vaccines**

Confirmation No.: (To be assigned)
Art Unit: (To be assigned)
Examiner: (To be assigned)
Atty. Docket:1530.0310003/EKS/EJH/ALS

Preliminary Amendment and Submission of Sequence Listing

Commissioner for Patents
Box Patent Application
Washington, D.C. 20231

Sir:

Applicant submits the following Preliminary Amendment, which is requested to be entered before examination. This Amendment is provided in the following format:

(A) A clean version of each replacement paragraph/section/claim along with clear instructions for entry;

(B) Starting on a separate page, appropriate remarks and arguments. 37 C.F.R. § 1.121 and MPEP 714; and

(C) Starting on a separate page, a marked-up version entitled: "Version with markings to show changes made."

Amendments

In the Specification:

Please insert the Sequence Listing at the end of the application.

Please replace paragraph [0009] on page 4 with the following paragraph:

One aspect of the present invention is an adjuvant composition comprising a mixture of one or more cytofectins and one or more co-lipids, which adjuvant composition is useful for enhancing the humoral immune response of a vertebrate to an immunogen. Preferably, the adjuvant composition includes the cytofectin GAP-DMORIE and one or more co-lipids. Preferably also, the co-lipid is a neutral lipid such as, for example, a phosphatidylethanolamine. More preferably, the co-lipid is 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and/or 1,2-dimyristoyl-glycero-3-phosphoethanolamine (DMPE). Most preferably, the co-lipid is DPyPE.

Please replace paragraph [0085] on page 31 with the following paragraph:

Single cell suspensions of splenocytes were pelleted and resuspended in RPMI 1640 medium containing L-glutamine and 25 mM HEPES and supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), 55 μM ß-mercaptoethanol and 10 % FBS. Unless otherwise noted, all tissue culture media and reagents were obtained from Gibco BRL Life Technologies (Rockville, MD). Then, 2.5 x 10^7 splenocytes were cultured for 5 days in 25 cm^2 tissue culture flasks in a total of 10 ml of media with $NP_{147-155}$ peptide (H-2K^d TYQRTRALV) (SEQ ID NO: 1) or β-$gal_{876-884}$ peptide (H-2L^d

TPHPARIGL) (SEQ ID NO: 2) at 1 μg/ml and recombinant murine IL-2 (Roche Molecular Biochemicals, Indianapolis, IN) at 0.5 U/ml.

Please replace paragraph [0086] on page 31 with the following paragraph:

For the CTL assay, P815 cells were labeled with 0.15 mCi $Na_2{}^{51}CrO_4$ (NEN Life Science Products, Boston, MA) in 30 μl saline at 37°C for 35 minutes. Labeled cells were pulsed with 20 μg NP peptide or β-gal peptide (H-2L^d TPHPARIGL) (SEQ ID NO: 2) in 1 ml RPMI 1640 media at 37°C for 40 minutes or were used unpulsed. Duplicate titrations of splenocytes were prepared by serially diluting the cells 1:3 in 96 well round bottom plates (ICN Biomedicals, Aurora, OH). Target cells were added at 1 x 10^4 cells/well in a final volume of 200 µl/well at the designated effector:target ratios (E:T). The plates were centrifuged and incubated for 4 hours at 37°C with 5 % CO_2. Counts per minute were determined for 100 µl of supernatant from each well. Specific lysis was calculated as % specific lysis = [(a-b)/(c-b)]100 where a is the average cpm released in the presence of effectors, b is the average cpm released from target cells incubated in media only and c is the cpm released from target cells in the presence of 1% Triton-X 100.

Please replace paragraph [0114] bridging pages 45-46 with the following paragraph:

Spleens were removed from euthanized mice at 11-12 weeks after the first injection, and 2.5 X 10^7 splenocytes were cultured for 5 days in 6 well plates in a total of 5 ml of RPMI 1640 medium (unless otherwise noted, all tissue culture reagents were

obtained from Gibco BRL Life Technologies, Rockville, MD) containing L-glutamine and 25 mM HEPES and supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), 5.5 x 10^{-5} M β-mercaptoethanol and 10% FBS (10% media) with either $NP_{147-155}$ peptide (H-2K^d TYQRTRALV) (SEQ ID NO: 1) or β-$gal_{876-884}$ peptide (H-2L^d TPHPARIGL) (SEQ ID NO: 2) at 1 μg/ml and recombinant murine IL-2 (Roche Molecular Biochemicals, Indianapolis, IN) at 0.5 U/ml.

In the Claims:

Please cancel claims 1-52 and 54-66 without prejudice or disclaimer thereof.

Remarks

Upon entry of the foregoing amendment, claim 53 is pending in the application. Claims 1-52 and 54-66 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein.

The specification has been amended to direct the entry of the Sequence Listing after the claims and to provide the SEQ ID NOs next to the specific sequence. In addition, at paragraph [0009] at the top of page 5, the term "1,2-dimyristoyl-glycer-3-phosphoethanolamine" has been replaced with "1,2-dimyristoyl-glycero-3-phosphoethanolamine" merely to correct a typographical error. Paragraph numbering has been added throughout the specification.

Applicant respectfully asserts that no new matter has been added by way of the above amendments, and that this application is now in condition for examination on the merits. In accordance with 37 C.F.R. § 1.821(g), this submission includes no new matter. In accordance with 37 C.F.R. § 1.821(f), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith in the above application are the same.

Summary

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided. Prompt and favorable consideration of this Preliminary Amendment and Submission of Sequence Listing is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Aaron L. Schwartz
Agent for Applicant
Registration No. 48,181

Date: March 19 2003

1100 New York Avenue, N.W.
Suite 600
Washington, D.C. 20005-3934
(202) 371-2600

Versions with markings to show changes made

In the Specification:

The Sequence Listing has been added to the application.

Paragraph [0009] on page 4 has been amended as follows:

One aspect of the present invention is an adjuvant composition comprising a mixture of one or more cytofectins and one or more co-lipids, which adjuvant composition is useful for enhancing the humoral immune response of a vertebrate to an immunogen. Preferably, the adjuvant composition includes the cytofectin GAP-DMORIE and one or more co-lipids. Preferably also, the co-lipid is a neutral lipid such as, for example, a phosphatidylethanolamine. More preferably, the co-lipid is 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE), and/or [1,2-dimyristoyl-glycer-3-phosphoethanolamine] 1,2-dimyristoyl-glycero-3-phosphoethanolamine (DMPE). Most preferably, the co-lipid is DPyPE.

Paragraph [0085] on page 31 has been amended as follows:

Single cell suspensions of splenocytes were pelleted and resuspended in RPMI 1640 medium containing L-glutamine and 25 mM HEPES and supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml), 55 µM ß-mercaptoethanol and 10 % FBS. Unless otherwise noted, all tissue culture media and reagents were obtained from Gibco BRL Life Technologies (Rockville, MD). Then, 2.5 x 10^7 splenocytes were

cultured for 5 days in 25 cm^2 tissue culture flasks in a total of 10 ml of media with $NP_{147-155}$ peptide (H-2K^d TYQRTRALV) (SEQ ID NO: 1) or β-$gal_{876-884}$ peptide (H-2L^d TPHPARIGL) (SEQ ID NO: 2) at 1 μg/ml and recombinant murine IL-2 (Roche Molecular Biochemicals, Indianapolis, IN) at 0.5 U/ml.

Paragraph [0086] on page 31 has been amended as follows:

For the CTL assay, P815 cells were labeled with 0.15 mCi $Na_2{}^{51}CrO_4$ (NEN Life Science Products, Boston, MA) in 30 μl saline at 37°C for 35 minutes. Labeled cells were pulsed with 20 μg NP peptide or β-gal peptide (H-2L^d TPHPARIGL) (SEQ ID NO: 2) in 1 ml RPMI 1640 media at 37°C for 40 minutes or were used unpulsed. Duplicate titrations of splenocytes were prepared by serially diluting the cells 1:3 in 96 well round bottom plates (ICN Biomedicals, Aurora, OH). Target cells were added at 1 x 10^4 cells/well in a final volume of 200 µl/well at the designated effector:target ratios (E:T). The plates were centrifuged and incubated for 4 hours at 37°C with 5 % CO_2. Counts per minute were determined for 100 µl of supernatant from each well. Specific lysis was calculated as % specific lysis = [(a-b)/(c-b)]100 where a is the average cpm released in the presence of effectors, b is the average cpm released from target cells incubated in media only and c is the cpm released from target cells in the presence of 1% Triton-X 100.

Paragraph [0114] bridging pages 45-46 has been amended as follows:

Spleens were removed from euthanized mice at 11-12 weeks after the first injection, and 2.5 X 10^7 splenocytes were cultured for 5 days in 6 well plates in a total of 5 ml of RPMI 1640 medium (unless otherwise noted, all tissue culture reagents were obtained from Gibco BRL Life Technologies, Rockville, MD) containing L-glutamine and 25 mM HEPES and supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), 5.5 x 10^{-5} M β-mercaptoethanol and 10% FBS (10% media) with either $NP_{147-155}$ peptide (H-2K^d TYQRTRALV) (SEQ ID NO: 1) or β-$gal_{876-884}$ peptide (H-2L^d TPHPARIGL) (SEQ ID NO: 2) at 1 μg/ml and recombinant murine IL-2 (Roche Molecular Biochemicals, Indianapolis, IN) at 0.5 U/ml.

In the Claims:

Claims 1-52 and 54-66 have been canceled.